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Take-off mechanisms in parasitoid wasps

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ABSTRACT

High speed video analyses of the natural behaviour of parasitoid wasps revealed three strategies used to launch themselves into the air. Which strategy is the most energy efficient? In *Pteromalus puparum*, 92% of take-offs by were propelled entirely by movements of the middle and hind legs which were depressed at their coxo-trochanteral and extended at their femoro-tibial joints. The front legs left the ground first, followed by the hind legs, so that the middle legs provided the final propulsion. Second, in other species of a similar mass, *Cotesia glomerata* and *Leptopilina boulardi*, all take-offs were propelled by a mean of 2.8 and 3.8 wingbeats respectively with little or no contribution from the legs. The first strategy resulted in take-off times that were four times shorter (5 versus 22.8 ms) and take-off velocities that were four times faster (0.8 versus 0.2 m s^{-1}). Calculations from the kinematics indicate that propulsion by the legs was the most energy efficient strategy, because more energy is put into propulsion of the body, whereas in take-off propelled by repetitive wing movements energy is lost to generating these movements and moving the air. In heavier species such as *Netelia testacea* and *Amblyteles armatorius*, take-off was propelled by the combined movements of the middle and hind legs and wingbeats. In *A. armatorius*, this resulted in the longest mean take-off time of 33.8 ms but an intermediate take-off velocity of 0.4 m s^{-1}. In all three strategies the performance could be explained without invoking energy storage and power amplification mechanisms.

INTRODUCTION

Three different strategies are used by a wide diversity of insects to launch a take-off from the ground and into the air. First, rapid movements of the legs propel jumping to achieve take-off, without any contribution from the wings. Second, repeated movements of the wings generate take-off with no obvious thrust contributed by the legs. The third strategy is to combine propulsive movements of both the legs and the wings. This paper analyses the dynamics of these different strategies for take-off and compares their energy efficiencies. We propose that generating take-off by a rapid jump propelled by the legs is more energy efficient than a take-off generated by repetitive flapping movements of the wings and could provide the basis of an explanation as to why so many winged insects jump to take-off. This hypothesis is tested by analysing the take-off mechanisms and performance of a five species of parasitoid wasps (Hymenoptera).

The most well-documented species of the Hymenoptera that jump are wingless ants. Trap jaw ants, *Odontomachus bauri*, use rapid closing movements of the mandibles to strike a hard substrate and propel a backwards escape jump (Patek et al., 2006) that increase the chance of surviving encounters with predatory insects such as antlions (Neuroptera) (Larabee and Suarez, 2015). They also use leg movements to jump forwards (Sorger, 2015). Ants such as *Polyrhachis laboriosa* that live in trees can jump downwards to escape predators or find new foraging sites (Mercier and Lenoir, 1999). Others like the wingless workers of *Cephalotes atratus* live higher in the canopy and either jump or fall but adjust their glide path by asymmetric movements of the hind legs and gaster to regain contact with the trunk of the same tree lower down (Yanoviak et al., 2005; Yanoviak et al., 2010). Other ants like the Indian ant *Harpegnathos saltator* jump by more conventional propulsive movements of the legs.

to escape predators, to catch prey in flight and to perform group movements that may aid in prey detection or predator avoidance (Musthak Ali et al., 1992). The jumping mechanism used is unclear. In one description the hind legs push first and then the rapid movements of the middle legs generate the final propulsion to modest take-off velocities of 0.7 m s^{-1} (Tautz et al., 1994). A second description indicates that propulsive movements of the middle and hind legs are synchronous, a conclusion that is said to be supported by the electrical activity of muscles in the middle and hind legs also being synchronous during "fictive" jumping movements (Baroni Urbani et al., 1994). Another species, *Myrmecia nigrocincta* is also reported to move its middle and hind legs synchronously to propel jumping (Tautz et al., 1994). *Gigantiops destructor* combines movements of the middle and hind legs with a forward rotation of the gaster that shifts the centre of mass of the body and could reduce body spin once airborne (Tautz et al., 1994).

Some larval wasps have abrupt movements that resemble jumping. The larvae of *Neuroterus saltatorius* (Cynipidae) develop in small galls on the leaves of oak trees. When these galls fall to the ground rapid contractions of the U-shaped larva within make the gall bounce 10 mm high (Manier and Deamer, 2014). Similarly, a larva of *Bathyplectes anurus* (Ichneumonidae) spins a cocoon within that of its larval weevil host and its twitch-like movements propel jumps some 50 mm high (Saeki et al., 2016).

Adult, winged wasps of a few species within the family (Eupelmidae) are more conventional but able jumpers propelled by movements of the legs (Gibson, 1986). No measurements of their performance have been reported, and mechanisms have instead been inferred from their thoracic morphology and musculature. The middle legs are thought to propel jumping and the necessary power is suggested to be generated by different mesothoracic muscles in males and females, although jumping appears to be similar in both sexes (Gibson, 1986). A pad of material onto which one of these muscles attaches has some of the properties of the elastic protein resilin, leading to the inference that the jumping mechanism must involve the storage of energy (Gibson, 1986). A mechanism, however, has not been demonstrated that would explain how energy is stored. These wasps unlike the wingless ants, but like many other insects such and moths (Burrows and Dorosenko, 2015a) and butterflies (Bimbard et al., 2013; Sunada et al., 1993), may use jumping as a means of launching into flight. This take-off objective might also be met by repetitive beating of the wings acting alone, or in concert with propulsive legs movements as in whiteflies (Ribak et al., 2016).

Why take-off with a jump powered by rapid leg movements rather than simply flapping the wings? To address this question, this paper analyses from high speed videos the take-off strategies and mechanisms of parasitoid wasps, selected for two reasons. First, these species fall into two groups based on their mass; three have masses of 1 mg or less, while the others have masses that are 50 times greater. We ask of all these species whether take-off is propelled by leg movements and if so which legs are used. Alternatively is take-off propelled by wing movements or by a combination of legs and wing movements? From these analyses we calculate which mechanisms are the most energy efficient and whether energy storage with concomitant power amplification has to be invoked to explain the observed take-off times and velocities.

MATERIALS AND METHODS

Five species of parasitoid wasps were analysed. 1. *Pteromalus puparum* (Linnaeus, 1758) (family Pteromalidae) (Fig. 1A). 2. *Cotesia glomerata* (Linnaeus, 1758), formerly *Apanteles glomeratus* (family Braconidae) (Fig. 1B). Both species were found parasitizing a culture of the cabbage white butterfly. 3. *Leptopilina boulardi*, (Barbotin, Carton & Kelner-Pillault, 1979) (family Figitidae) (Fig. 1C,D) was found in a *Drosophila* culture. 4. *Amblyteles armatorius* (Forster, 1771) (Fig. 1E) and 5. *Netelia testacea* (Gravenhorst, 1829) (family Ichneumonidae) were caught amongst garden plants in Girton, Cambridge, UK. The phylogenies of these wasps within the Hymenoptera have recently been analysed (Branstetter et al., 2017; Quicke et al., 2012).

Live wasps were photographed with a Nikon D7200 camera fitted with a 100 mm Nikon macro lens. The anatomy of the legs was examined in intact wasps, and in those fixed and stored in 70% alcohol, or 50% glycerol. Leg lengths were measured to an accuracy of 0.1 mm from images taken with a GX CAM 5-C camera (GT Vision Ltd., Stansfield, Suffolk, UK) attached to a Leica MZ16 microscope (Wetzlar, Germany) and projected onto a monitor (Table 1). Body masses were determined to an accuracy of 0.1 mg with a Mettler Toledo AB104 balance (Beaumont Leys, Leicester, UK).

Sequential images of take-offs were captured with a single Photron Fastcam SA3 camera (Photron (Europe) Ltd, High Wycombe, Bucks., UK) fitted with a 100 mm micro Tokina lens. Frame rates of 1000 s^{-1} with an exposure time of 0.2 ms and 5000 s^{-1} with an exposure time of 0.1 ms were used. Several of the three smaller species were placed at the same time in a chamber made of optical quality glass that was 25 mm wide, 25 mm tall and 12 mm deep. The two larger species were placed individually in a larger chamber measuring 80 mm wide, 80 mm tall and 25 mm deep. The camera pointed directly at the middle of these chambers and focused on an individual wasp. The floor, side walls and ceiling of each chamber were made of 12 mm thick, closed cell foam (Plastazote, Watkins and Doncaster, Cranbrook, Kent, UK) from which the wasps would take-off spontaneously. Tracks of the movements of specific body parts were made manually frame by frame with Tracker software (http://physlets.org/tracker/); auto-tracking failed to follow the movements of small body parts reliably. The frame at which a particular leg lost contact with the ground was determined by playing the video backwards and forwards frame by frame. An abrupt shift in position of a tarsus indicated that it was no longer load bearing and had lost contact with the ground. Take-off was indicated by the last leg losing contact with the ground so that the insect was airborne; it was designated as time t = 0 ms. The acceleration time was defined as the period from the first detectable movement of the propulsive legs, or wings, until take-off. Peak velocity was calculated as the distance moved in a rolling 3 point average of successive images before take-off. A point on the body that could be recognized in successive frames and was close to the centre of mass was selected for measurements of the trajectory. The angle subtended by a line joining these positions after take-off, relative to the natural horizontal, gave the trajectory angle. The body angle was defined as the angle subtended by the longitudinal axis of the wasp relative to the natural horizontal at take-off. The results are based on high speed videos of 292 take-offs by 35 wasps from at temperatures of 23-25º C. At least three jumps were analysed in detail for each individual wasp. The number of specimens of *N. testacea* was small, so that no quantitative analysis was performed. Data are presented as means ± standard error of the mean (s.e.m.) for an

individual insect and as grand means (mean of means) for all the individuals of a particular species.

RESULTS

Body Form

The parasitoid wasps fell into two distinct groups on the basis of their mass and size. First were three small species *L. boulardi*, *P. puparum* and *C. glomerata* with body masses of respectively 0.4 ± 0.03, 1.0 ± 0.1 and 1.0 ± 0.07 mg and body lengths of 1.8 ± 0.06, 2.7 ± 0.01 and 3.1 ± 0.05 mm (N=7 for each species) (Table 1). By contrast, the second group contained two heavier and larger species, *A. armatorius* and *N. testacea*, with *A. armatorius* having a mass of 56.9 ± 0.83 mg and a body length of 19.5 ± 0.5 mm (N=7).

In all species the middle legs were either the same length as the front legs (as in *L. boulardi)*, or they were just 20% longer. The middle legs were also short relative to the body length, ranging from 46% in *A. armatorius* to 68% in *P. puparum* (Table 1). The hind legs in *L. boulardi* and *P. puparum* were 40% longer than the front legs, in *C. glomerata* they were 70% longer and in *A. armatorius* they were 80% longer. Relative to body length, the hind legs were longest at 87% in *C. glomerata* and shortest at 70% in *A. armatorius* (Table 1). The middle femora were 12% wider than those of the front legs in *C. glomerata* but in *A. armatorius* they were only 3% wider (Fig. 1F,G). By contrast, the hind femora of these two species were 74-75% wider than those of the front legs.

Kinematics of jumping and take-off

Pteromalus puparum

In *P. puparum* 65 of 71 jumps (91.55%) performed by 11 individuals were propelled by movements of the middle legs alone (Figs 2,3, Supplementary material Movie 1). The wings remained closed so that the forward and upward trajectory were determined solely by the forces generated by the legs during the jump. In some jumps, the wings opened after take-off and flapping flight ensued, but this rarely occurred in the experimental conditions.

In a selected jump of *P. puparum* viewed from the side (Fig. 2), the hind legs were the first to move some 4 ms before take-off and their contribution to propulsion was brief because they were the first pair of legs to lose contact with the ground 0.6 ms later followed by the front legs 2 ms before take-off (Fig. 2A). During this time the two middle legs moved symmetrically by depressing about their coxo-trochanteral joints and extending about their femoro-tibial joints. These movements continued until take-off so that the middle legs alone were then responsible for propulsion. After take-off, the left and right middle legs crossed because the forces they generated now acted only against the air. Clear differences in when the three pairs of legs lost contact with the ground were revealed by plotting their movements against time (Fig. 2B). The sequence in which the legs moved and lost contact with the ground varied, however, both between successive jumps of an individual and between jumps of different individuals; the hind legs were usually the first to lose contact with the ground, but sometimes the front legs were the first. Different sequences did not correlate with the angle of the body relative to the ground, or with the angle of the jump trajectory after take-off. The action of the middle legs was a constant feature in all jumps; their joints

depressed and extended progressively to provide upwards and forwards propulsion, and their tarsi were always the last to lose contact with the ground.

Six jumps by *P. puparum* (8.45% of the total jumps by this species) were propelled by the same leg movements but were also accompanied before take-off by a single depression of the wings (Fig. 3, Supplementary material Movie 2). The wings opened 30 ms before take-off and reached their fully open and elevated position before the first leg movement occurred. The wings then began to depress while the front legs were the first to lose contact with the ground, followed by the hind legs. At take-off, the wings were completing their first depression movement. After take-off the wings began to beat so that there was a seamless transition to powered flight. The mean wingbeat frequency in these wasps once airborne was 185.4 ± 3.2 Hz so that the period from one full elevation to the next was 5.4 ± 0.1 (N = 10 individuals) (Table 2).

Cotesia glomerata

Take-off by *C. glomerata* was propelled by a series of wingbeats with only small movements of the middle and hind legs occurring (Fig. 4, Supplementary material Movie 3). Preparation for take-off was marked by the adoption of a steep angle of the body relative to the ground (69.1 ± 8.0 degrees) (Table 3) so that the front legs were lifted from the ground. The wings then opened and executed 2.8 ± 0.3 wingbeats before take-off (21 take-offs, N=3 individuals) (Fig. 4A). The mean wingbeat frequency was 126.1 ± 6.7 Hz (period 8.2 ± 0.3 ms) (Table 3). The first wingbeat before take-off was often of small amplitude but thereafter all wingbeats were of a full amplitude during both the acceleration phase of take-off and once airborne (Fig. 4B-D). At the start of the first wing movement the middle and hind legs began to show small changes in the angles between the body and the femora and between the femora and tibiae (Fig. 4A,D). These changes continued after take-off as the legs sagged beneath the body under the influence of gravity, suggesting that the initial small changes observed before take-off might be due to the lift generated by the wings rather than active propulsion by the legs. The hind legs were the next to lose contact with the ground so that as in *P. puparum*, it was the middle legs that finally lost contact at take-off while the wings were being depressed (Fig. 4D).

Leptopilina boulardi

In *L. boulardi*, the lightest of all the species analysed, propulsion for take-off shifted further towards a greater reliance on the wings. Preparation for take-off was initiated by tilting of the front of the body upwards so that the front legs lost contact with the ground and thus made no further contribution. The wings then opened and executed 3.8 ± 0.1 wingbeats (28 take-offs, N=4 individuals) with take-off again being accomplished during wing depression (Figs 5, 6). The mean wingbeat frequency was the highest at 198.9 ± 5.4 Hz (mean period 5.0 ± 0.1 ms (N= 4 individuals)) (Table 2). No contribution to propulsion could be discerned by the middle and hind legs as viewed from directly in front (Fig. 5A) or from the side (Fig. 6, Supplementary material Movie 4). The angles between the body and femora, and between the femora and tibiae in both pairs of these legs did not change before take-off, but once airborne they increased as the legs sagged beneath the body (Fig. 5B, C). The wing movements described symmetrical paths leading to take-off while the movements of the body described an upward trajectory with a mean angle of 91.2 ± 31.8 degrees but with much variation (Fig. 5B, Table 3). The rhythmic wing movements continued after take-off so that there was a smooth transition to powered flight.

Ichneumonidae

A. armatorius and *N. testacea*, were the largest wasps analysed and both used a combination of leg and wing movements to propel take-off (Figs 7, 8). The legs were, however, used in a different sequence to that described for the preceding wasps. The wings opened first and then before they started to beat (Fig. 7A, Supplementary material Movie 5), or during their first depression (Fig. 8A), the hind legs began their propulsive movements. The front legs were the first to leave the ground followed by the middle legs (Figs 7B, 8B). The hind legs were thus the only legs adding to the forces during the latter part of the propulsive phase of the take-off. The wings generated a mean of 2.6 ± 0.2 wingbeats (27 take-offs) before take-off lowest mean frequency for any of the wasps recorded here of 80 ± 3.7 Hz (mean period 13.1 ± 0.5) (N= 9 individuals) (Table 2). Both the angle between the body and the hind femora and between the femora and the tibiae changed progressively to depress and extend the hind legs to take-off while the wings were beating (Fig. 7B). For *N. testacea* plotting the trajectories described by the tip of the right front wing before take-off together with the movements of the tarsi of the right middle and hind legs showed that take-off occurred during the depression phase of a wingbeat and that the middle legs lost contact with the ground well before the hind legs (Fig. 8B).

Jumping and take-off performance

Jumping and take-off performance were derived from measurements taken from the high speed videos, and from subsequent calculations (Table 3). The time taken to accelerate to take-off varied by a factor of six between the different species. It was shortest at 5.0 ± 0.3 ms (three jumps by each of 11 wasps) in *P. puparum*, which was propelled by leg movements. The acceleration times of take-offs by this species that were assisted by a single depression of the wings all fell within the range of times shown in jumps propelled only by the legs, suggesting only a small contribution of the wings to lift. In other species where take-off was propelled by a few wingbeats, the take-off time was longer at 19.3 ± 1.1 ms (N=4 individuals) in *L. boulardi* and 22.8 ± 2.8 ms (N=3 individuals) in *C. glomerata*. It was longest at 33.8 ± 1.9 ms (N=9 individuals) in the heaviest wasp *A. armatorius* that was propelled by combined leg and wing movements.

Take-off velocity varied by a factor of four between the different species. The fastest velocities, mean (0.8 ± 0.1 m s^{-1}) and best (0.99 m s^{-1}), were achieved by *P. puparum*. Both *L. boulardi* and *C. glomerata* which have a similar mass, but were propelled by wing movements, could only achieve mean take-off velocities that were a quarter of this at 0.2 m s^{-1} (Table 3). The heaviest wasp *A. armatorius* that used leg and wing movements had an intermediate take-off velocity with a mean of 0.4 ± 0.03 m s^{-1} and best value of 0.6 m s^{-1}.

The trajectory of the take-off and the angle of the body relative to the horizontal were correlated with the propulsive strategy that was used. Where the legs provided the sole or main propulsion, as in *P. puparum*, the mean trajectory angle was 65.8 ± 7.8 degrees and where leg movements were combined with wing movements, as in the much heavier *A. armatorius*, the angle was shallower at 54.4 ± 4.9 degrees (Table3). The body angle relative to the ground was also low at 35.1 ± 4.6 and 25.4 ± 2.6 degrees respectively. In contrast, where wing movements were the sole or dominant propulsive force, the trajectories were much steeper; in *L. boulardi* they were almost vertical (mean 91.2 ± 5.4 degrees), and in *C. glomerata* they were backwards (mean

110.7 ± 14.6 degrees). The angle of the body at take-off was also steeper at 63.3 ± 10.2 degrees and 69.1 ± 8.0 degrees respectively.

The acceleration experienced at take-off was highest at 17 **g** in *P. puparum* which had the fastest take-off velocity and shortest acceleration time (Table 3). By contrast, in species with both slower take-off velocities and longer acceleration times, the values ranged only between 1-2 **g**.

The energy for take-off (E) was calculated in the same way (Equation 1) for each of the three strategies used by these parasitoid wasps

(1) $E = 0.5\ m\ v^2$

Where m = mass, kg
v = velocity, m s^{-1}

In *P. puparum* the mean energy required for a jump propelled by leg movements was 0.3 µJ and the mean power was 0.06 mW. In the two other species of similar mass, but in which take-off was propelled by wing movements the mean energy required was only 0.01- 0.02 µJ and the mean power 0.0003 - 0.001 mW. In the much heavier *A. armatorius*, these values were 10.9 µJ and 0.15 mW in its best jumps.

The power requirements for take-off in its best jumps by *P. puparum* were 766 W kg^{-1} muscle whereas in the remaining three species they were dramatically lower at 13 - 24 W kg^{-1} muscle in their best jumps. This calculation assumes that the muscles used to propel take-off comprised 10% of body mass.

DISCUSSION

Consequences of different strategies for take-off

This paper has shown that different species of parasitoid wasps use different strategies to generate take-off. In three species of similar mass (0.4 – 1.0 mg), two distinct strategies were found even in species that parasitise the same host. *P. puparum* propelled 92% of its jumps by rapid leg movements alone with no accompanying movements of the wings. In the small number of their remaining jumps, the wings executed just one depression movement of the wings before take-off which did not increase take-off velocity above that achieved by the propulsive leg movements alone. By contrast, in *C. glomerata* and *L. boulardi* wings movements were respectively either the main, or only contributors of force to take-off. In the much heavier *A. armatorius* (mass 50 times greater) a combination of propulsive leg movements and wing beating generated take-off. Only one species showed evidence of being able to change strategies. In a few take-offs, *P. puparum* added one cycle of wing depression to the same sequence of leg movements. The measurements were made in a limited behavioural context in which the take-offs could not be related directly to a particular sensory stimulus. Some take-offs appeared to be “spontaneous” but the possibility remains that others might have been triggered by the presence of members of the same species in the chamber at the same time. In insects such as *Drosophila* different take-off strategies are clearly used in different behavioural contexts (Card and Dickinson, 2008; Trimarchi and Schneiderman, 1995; von Reyn et al., 2014).

The following conclusions can be drawn about the effectiveness of the strategies for take-off, particularly by comparing the performance of the three species with similar, low masses. The shortest acceleration time of 5 ms and a mean take-off velocity of 0.8 m s^{-1}, with the fastest take-off reaching almost 1 m s^{-1}, was achieved by take-off propelled by movements of the legs in *P. puparum*. By contrast, in two other species of similar mass, take-off generated by beating the wings a mean of 2.8 times in *C. glomerata* and 3.8 times in *L. boulardi* resulted in acceleration times that were four times longer at 19-23 ms and take-off velocities that were four times slower at 0.2 m s^{-1}. The take-off angles and the subsequent trajectories of these two species once airborne were also much steeper than those of *P. puparum* which was propelled only by jumping movements of the legs. These three species of wasps had bodies of similar size, shape and mass. The legs of *P. puparum* and *L. boulardi* were also of similar proportions relative to each other and to body length. The exception was *C. glomerata* the hind legs of which were 70% longer than the front legs and the longest relative to body, but this increased leverage did not apparently contribute to take-off.

To calculate the energy expended in translation of the body, the same method was used for take-offs generated by both strategies. This method calculates the energy put into propelling the body into the air. Take-offs of *P. puparum* generated by leg movements resulted in 0.3 μJ of energy being put into achieving this goal, whereas in take-offs generated by flapping movements of the wings, this value fell by 15-30 times to 0.02 or 0.01 μJ. The reason for this dramatic difference is that in the latter strategy, energy is lost in generating repetitive movements of the wings and in moving air rather than in propelling the body upwards and forwards. These data therefore support the hypothesis of this paper that jumping as a mechanism to propel take-off is more energy efficient than propulsion generated by flapping movements of the wings. This energy efficiency is thus a further advantage conferred by jumping in addition to a shorter acceleration time to take-off and a faster take-off velocity.

This conclusion is even more striking when consideration is given to the mechanical power that is transmitted to the body during take-off. In all strategies, the relevant muscles must generate much power but the amount seen in the output kinematics is very different for the different strategies. In take-offs by *P. puparum*t that are propelled by leg movements, this power is almost completely transferred to the final movement of the wasp (Table 3). The high power outputs of this species may also indicate a contribution from hind leg muscles to take-off. By contrast, in C. *glomerata*, L. *boulardi*, and A. *amartorius* in which take-offs are propelled by wing movements, less than 10% of the power generated by the muscles is transferred directly to the final movement of the wasp (Table 3). Most of the power is used in accelerating the wings and the air beneath the wings, and not into accelerating the wasp itself.

In the heavier species such as *A. armatorius*, both leg and wing movements were combined to propel take-off in the longest acceleration time of 34 ms and to a take-off with a velocity of 0.4 m s^{-1} that is half generated by *P. puparum* when jumping. Lifting the heavy body must contribute to this long acceleration time and lower take-off velocity and offers a possible explanation of why a combination of leg and wing movements are needed to effect take-off.

Take-off performance

How does the jumping performance of the wasps analysed here compare with other insects that also use a power generating mechanism that depends on direct contractions of leg muscles and four propulsive legs? The take-off velocity of *P.*

puparum matched that of lacewings (Neuroptera) 0.5 - 0.6 m s^{-1} (Burrows and Dorosenko, 2014) and moths (Lepidoptera) 0.6 - 0.9 m s^{-1} (Burrows and Dorosenko, 2015a), overlapped with some caddis flies (Trichoptera) 0.7 - 1.0 m s^{-1} (Burrows and Dorosenko, 2015b), but fell short of the velocities achieved by a praying mantis (Mantodea) 1.0 m s^{-1} (Burrows et al., 2015) and the fly *Hydrophorus alboflorens* (Diptera) 1.6 m s^{-1} (Burrows, 2013a). Two of the species of wasps analysed here, *C. glomerata* and *L. boulardi*, used the wings alone to propel take-off and reached low velocities of 0.2 m s^{-1}. Their take-offs were almost vertical or sometimes slightly backwards so their trajectories were much steeper than those of wasps that are propelled by the legs alone or assisted by the wings. Caddis flies also differ in their take-off trajectory depending on the strategy they use (Burrows and Dorosenko, 2015b).

Use of two pairs of legs for take-off

A number of jumping solutions have evolved in insects particularly when the legs are used for propulsion. When one pair of legs is used by true flies (Diptera) it is the middle legs (Hammond and O'Shea, 2007; Trimarchi and Schneiderman, 1995b), but in other insects it is more frequently the hind legs. In bush crickets these legs are long relative to the body and allow jumps to be propelled by direct contractions of the muscles acting on these long levers (Burrows and Morris, 2003). Higher take-off velocities have been achieved by catapult mechanisms involving the hind legs in insects such as grasshoppers, fleas and froghoppers (Bennet-Clark, 1975; Bennet-Clark and Lucey, 1967; Burrows, 2003). In catapult mechanisms, energy can be stored in advance of a jump to enable power to be amplified with a concomitant reduction of the acceleration time. The stored energy is then released suddenly to generate fast leg movements that propel a jump. So rapid are the leg movements of frog-, plant- and tree-hoppers (Burrows, 2006; Burrows, 2009; Burrows, 2013b) using this mechanism that additional specialisation are needed to ensure that the leg movements are synchronised (Burrows, 2010; Burrows and Sutton, 2013) so as not to result in rotation of the body and the loss of energy for forward momentum.

In other insects, such as the wasps described here, both the middle and hind legs can potentially be used to propel jumping or assist in take-off. The front legs can be excluded as in none of the recorded take-offs do they move in ways that are consistent with generating propulsion. In snow fleas (Burrows, 2011), praying mantis (Burrows et al., 2015) and the Dolichopodid fly *Hydrophorus alboflorens* (Burrows, 2013a) the middle and the hind legs leave the ground at the same time so both are able to contribute thrust during the last part of the acceleration phase of take-off. In caddis flies (Trichoptera) (Burrows and Dorosenko, 2015b) the middle legs are the last to leave the ground, but in moths (Lepidoptera) (Burrows and Dorosenko, 2015a), lacewings (Neuroptera) (Burrows and Dorosenko, 2014) and mirid bugs (Hemiptera) (Burrows and Dorosenko, 2017) it is the hind legs. In the wasp *P. puparum*, the middle legs are the last to leave the ground so that it is using the same strategy as caddis flies (Burrows and Dorosenko, 2015b). By contrast, the hind legs of *A. armatorius* are the last to lose contact with the ground so that it is using the same strategy as ants (Baroni Urbani et al., 1994; Tautz et al., 1994), moths (Burrows and Dorosenko, 2015a) and lacewings (Burrows and Dorosenko, 2014).

What advantages does the use of two propulsive pairs of legs confer? First, four propulsive legs ensure that forces needed for take-off are distributed over a larger surface area of the substrate. This could allow take-off from more compliant surfaces. Adding thrust from wing movements should further reduce the forces transmitted

directly to the ground, while at the same time allowing a smooth transition into forward, powered flight.

A second advantage is that the muscles of both pairs of legs power the movement, effectively almost doubling the muscle mass available for take-off. Estimates that the jumping muscles represent about 10% of total body mass are based on measurements from insects using just one pair of propulsive legs. On this basis, the calculated energy requirements for take-off by the wasps studied here are low and only in *P. puparum* do they reach the high end of values shown for muscle from a range of animals (Askew and Marsh, 2002; Ellington, 1985; Josephson, 1993; Weis-Fogh and Alexander, 1977). Increasing the muscle mass used for jumping would thus reduce the expected energy demands to well within these limits and allow all the wasp jumps and take-offs to be explained by direct contractions of the muscles. Mechanisms involving energy storage have been invoked (Gibson, 1986) to explain the take-offs of some wasps, but the data presented here indicates that such mechanisms are not required for the performance of the parasitoid wasps studied here. A clear disadvantage of using four legs is that it precludes achieving the high take-off velocities that catapult mechanisms can generate, because no neural or mechanical mechanisms have been found that could synchronise all these legs with the necessary precision.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

No competing interests declared.

FUNDING

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AUTHOR CONTRIBUTIONS

M.B. initiated the project, carried out the experimental work and prepared the first draft of the paper. M.D. analysed the videos to determine take-off performance and contributed to subsequent versions of the manuscript.

Table 1. Body form of parasitoid wasps

Body length and mass, and lengths of the hind femora and tibiae (grand means ± s.e.m.) of four species of parasitoid wasps (N=7 for each species). The ratios of leg lengths are given relative to the front legs.

	Body Mass, mg	**Body Length, mm**	**Hind leg, Femur, mm**	**Hind leg, Tibia, mm**		**Ratio of leg lengths**		**Middle leg length as % of body length**	**Hind leg length as % of body length**	**Hind leg length (mm)/ body mass ^ 1/3 (mg)**
					Front	**Middle**	**Hind**			
Pteromalus puparum	1.0 ± 0.10	2.7 ± 0.01	0.6 ± 0.02	0.7 ± 0.03	1	1.2	1.4	68	78	2.1
Cotesia glomerata	1.0 ± 0.07	3.1 ± 0.05	0.7 ± 0.03	0.8 ± 0.03	1	1.2	1.7	59	87	1.6
Leptopilina boulardi	0.4 ± 0.03	1.8 ± 0.06	0.4 ± 0.03	0.5 ± 0.01	1	1	1.4	61	84	1.6
Amblyteles armatorius	56.9 ± 0.83	19.5 ± 0.50	3.4 ± 0.05	3.9 ± 0.09	1	1.2	1.8	46	70	3.6

Table 2. Wingbeat frequency and period

The number of wingbeats before take-off, the wingbeat frequency and period for four species of parasitoid wasps analysed in detail. N = number of individuals of each species, n = the total number of wingbeats measured; grand means (± s.e.m.) for each species. At least 3 wingbeats were measured after take-off for each individual wasp.

Species	**Number of wingbeats before take off**	**Wingbeat frequency, Hz**	**Wingbeat period, ms**
Pteromalus puparum (N = 10, n = 30)	Single depression	185.4 ± 3.2	5.4 ± 0.1
Cotesia glomerata (N = 3, n = 21)	2.8 ± 0.3	126.1 ± 6.7	8.2 ± 0.3
Leptopilina boulardi (N = 4, n = 28)	3.8 ± 0.1	198.9 ± 5.4	5.0 ± 0.1
Amblyteles armatorius (N = 9, n = 27)	2.6 ± 0.2	80.0 ± 3.7	13.1 ± 0.5

Table 3. Jumping performance of parasitoid wasps

Data in columns 2–6 are the grand means (± s.e.m.) for the measured jumping performance of each of the four species; the best performance (defined by the fastest take-off velocity) of a particular individual is also given. The values in columns 7–12 on the right are calculated from these measured data. N = number of individuals of each species that were analysed

	Body Mass	**Time to take off**	**Take-off velocity**	**Take-off angle**	**Body angle at take-off**	**Acceleration**	**g force**	**Energy**	**Power**	**Force**	**Power/kg muscle**
Formula	***m***		**v**			**f= v/t**	***g*= f/9.81**	**E=0.5 *m* v^2**	**=E/t**	**=*m* f**	**=power / (.1*m*)**
Units	**mg**	**ms**	**m s^{-1}**	**degrees**	**degrees**	**m s^{-2}**	***g***	**uJ**	**mW**	**mN**	**W kg^{-1}**
Pteromalus puparum											
Average (N=11)	1.0 ± 0.1	5.0 ± 0.3	0.8 ± 0.1	65.8 ± 7.8	35.1 ± 4.6	163	17	0.3	0.06	0.2	656
Best	0.7	6.4	0.99	67.8	37.5	155	16	0.3	0.05	0.1	766
Cotesia glomerata											
Average (N=3)	1.0 ± 0.07	22.8 ± 2.8	0.2 ± 0.01	110.7 ± 14.6	69.1± 8.0	9	1	0.02	0.001	0.01	10
Best	0.8	22.0	0.2			11	1	0.02	0.001	0.01	13
Leptopilina boulardi											
Average (N=4)	0.4 ± 0.03	19.3 ± 1.1	0.2 ± 0.02	91.2 ± 31.8	63.3 ± 10.2	9	1	0.01	0.0003	0.003	8
Best	0.4	14.2	0.2			17	2	0.01	0.0008	0.007	20
Amblyteles armatorius											
Average (N=9)	56.9 ± 0.83	33.8 ± 1.9	0.4 ± 0.03	54.4 ± 4.9	25.4 ± 2.6	12	1	4.7	0.14	0.7	24
Best	60.6	74.0	0.6	39.1	20.7	8	1	10.9	0.15	0.5	24

FIGURE LEGENDS

Fig. 1

Body and leg structure of four species of parasitoid wasps. (A -E) Photographs of four species analysed. (A) Side view of *Pteromalus puparum*. (B) Ventral view of *Cotesia glomerata*. (C,D) Side views of a female *Leptopilina boulardi* (C) with a large abdomen and a male (D) with long antennae. (E) Side view of *Amblyteles armatorius*. (F,G) Photographs of the right front, middle and hind legs viewed laterally of *C. glomerata* (F) and *A. armatorius* (G).

Fig. 2

Take-off by *Pteromalus puparum* propelled only by movements of the legs. (A) Selected images of a jump from the horizontal and to the right and viewed from the side are arranged in two columns with the timing of the frames given relative to take-off at time = 0 ms. Images were captured at 5000 s^{-1} and with an exposure time of 0.1 ms. In this and subsequent Figures, the front legs (LF, left front; RF, right front) are indicated by arrows with yellow heads, the middle legs (LM, RM) by arrows with white heads, and the hind legs (LH, RH) by arrows with pink heads. The triangles in the bottom left hand corners of each image indicate a constant spatial reference point. (B) Plots of the movements, against time, of the tarsi of the front, middle and hind legs during the same jump. The front and hind legs lost contact with the ground during the acceleration phase of the jump so that only the middle legs provided the final propulsion to take-off. The wings did not move.

Fig. 3

Take-off by *Pteromalus puparum* propelled by the legs and one depression of the wings. Selected images of a jump from the horizontal and viewed from the side were captured at 5000 s^{-1} and with an exposure time of 0.1 ms. They are arranged in three columns.

Fig. 4

Take-off by *Cotesia glomerata* propelled by movements of the wings. (A) Selected images captured at 1000 s^{-1} and with an exposure time of 0.2 ms are arranged in two columns. Take-off was from the horizontal and was viewed from the side. The wingbeats are counted from the peaks of elevation and in this and Figs 5, 6 and 8 are indicated in blue. (B,C) Tracks of the movements of the tip of the right front wing (cyan) and of the tarsi of the right middle (white) and right hind legs (pink). In (B) these tracks are superimposed on the image at the start of the first wing depression and in (C) on an image when airborne. The start positions of each track are marked by a large filled circle and the end positions by an **X**. (D) Plot of the movement of the tip of the right front wing, and of the angular changes in particular joints of the right middle and hind legs, against time.

Fig. 5

Take-off by a male *Leptopilina boulardi* propelled by movements of the wings. (A) Selected images of a take-off captured at 5000 s^{-1} and with an exposure time of 0.1 ms are arranged in four columns. The take-off was from the horizontal and towards the camera so that the wasp is viewed head-on. (B) Tracks of the movements of the tips of

the left (green) and right (cyan) hind wings and of the tarsi of the left (blue) and right (white) middle legs. The tracks are superimposed on an image of the wasp at the start of the acceleration phase. The large coloured circles indicate the initial positions, the black dots the positions at take-off and **X** the positions when airborne. (C) Plots against time of the same body parts during the same jump.

Fig. 6

Take-off by a female *Leptopilina boulardi* propelled by movements of the wings. This take-off was from the horizontal and is viewed from the side. Selected images captured at 5000 s^{-1} and with an exposure time of 0.1 ms are arranged in three columns.

Fig. 7

Take-off by *Amblyteles armatorius* propelled by movements of the wings and legs. (A) Selected images of a take-off viewed from the side were captured at 1000 s^{-1} and with an exposure time of 0.2 ms and are arranged in two columns. (B) Movement of the tip of the right front wing (cyan), and the changes in the angle between the body and the femur (pink triangles), and between the femur and tibia (pink squares) of the right hind leg are plotted against time. The propulsive movements of the hind legs began before the wing movements. Take-off occurred during depression of the second wingbeat.

Fig. 8

Take-off by *Netelia testacea* propelled by movements of the wings and legs. (A) Selected images of a take-off viewed from the side were captured at 1000 s^{-1} and with an exposure time of 0.2 ms and are arranged in two columns. Tracks of the movements of the tip of the right front wing (cyan) and the tarsi of the right middle (white) and right hind (pink) legs are superimposed on the image at take-off. (B) Plot of the movements of the same body parts during the same jump against time. Take-off occurred during depression of the third wingbeat.

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Figure 1

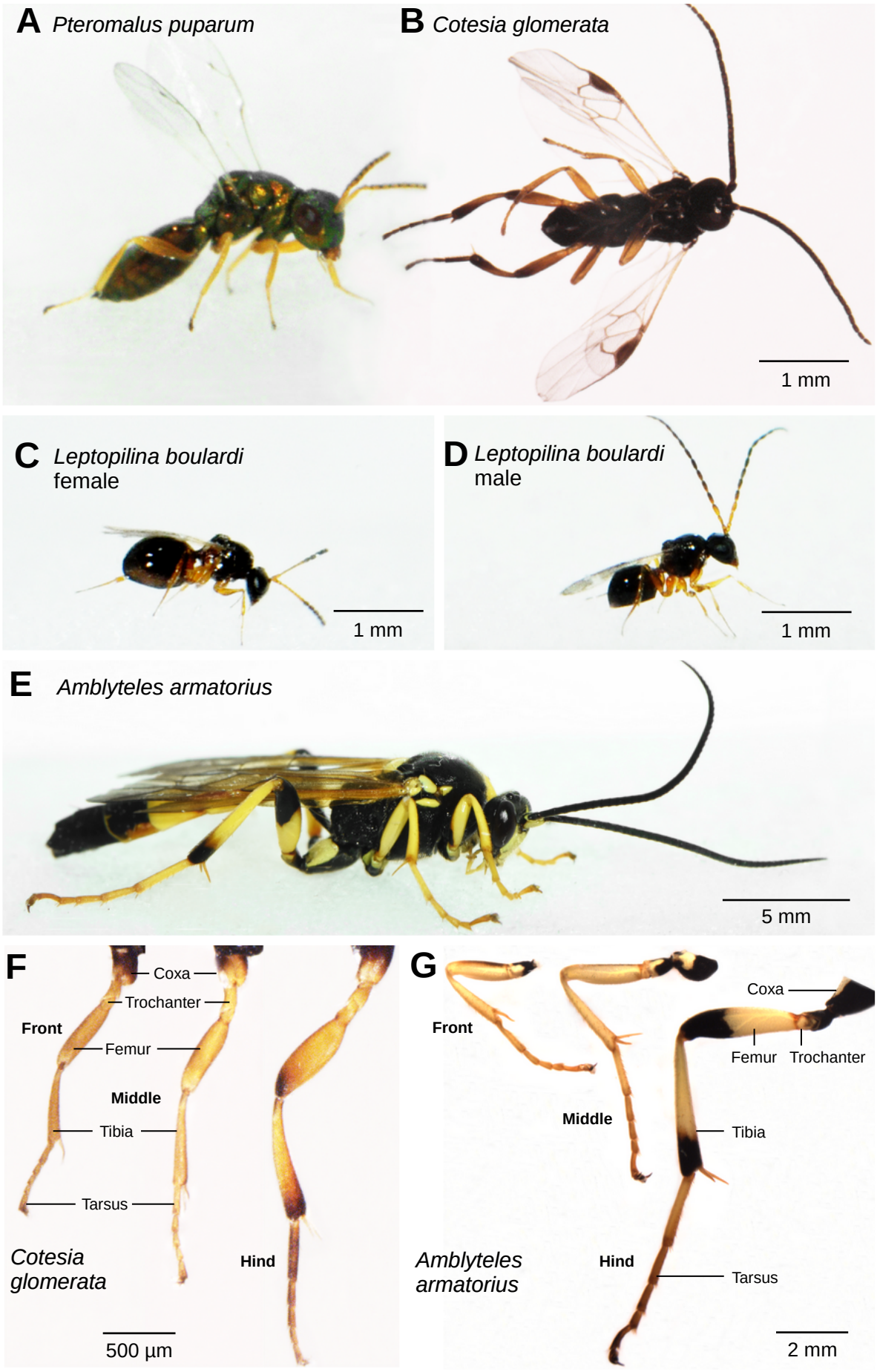
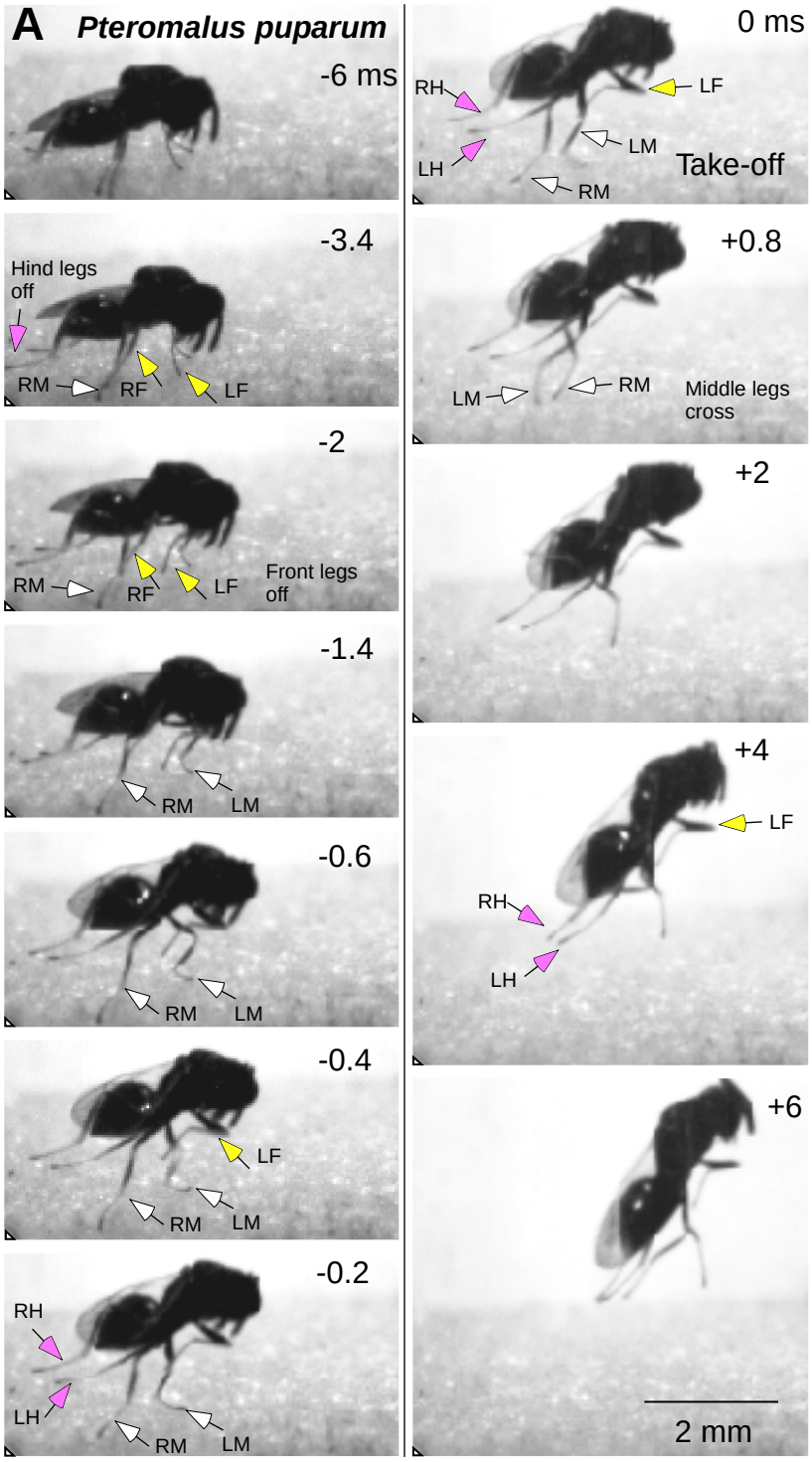

A Pteromalus puparum
B Cotesia glomerata
1 mm
C Leptopilina boulardi
female
1 mm
D Leptopilina boulardi
male
1 mm
E Amblyteles armatorius
5 mm
F
Coxa
Trochanter
Front
Femur
Middle
Tibia
Tarsus
Hind
Cotesia
glomerata
500 µm
G
Front
Coxa
Femur
Trochanter
Middle
Tibia
Hind
Tarsus
Amblyteles
armatorius
2 mm

Figure 2


A
Pteromalus puparum
-6 ms
0 ms
RH
LF
LM
LH
RM
Take-off
-3.4
Hind legs off
RM
RF
LF
+0.8
LM
RM
Middle legs cross
-2
RM
RF
LF
Front legs off
+2
-1.4
RM
LM
-0.6
RM
LM
+4
LF
RH
LH
-0.4
LF
RM
LM
+6
-0.2
RH
LH
RM
LM
2 mm

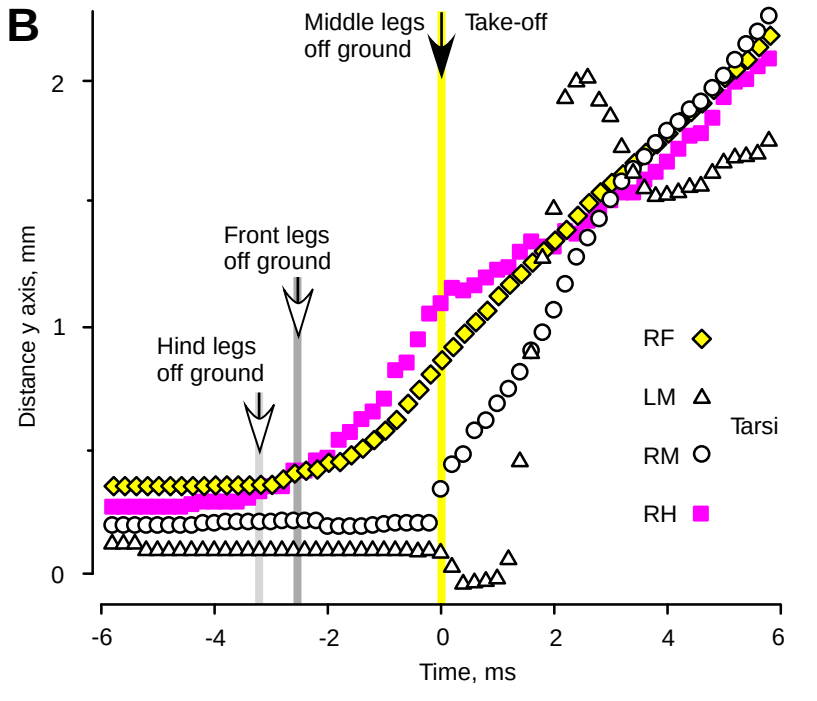

B
Middle legs off ground
Take-off
Front legs off ground
Hind legs off ground
Distance y axis, mm
0
1
2
-6
-4
-2
0
2
4
6
Time, ms
RF
LM
RM
RH
Tarsi

Figure 3

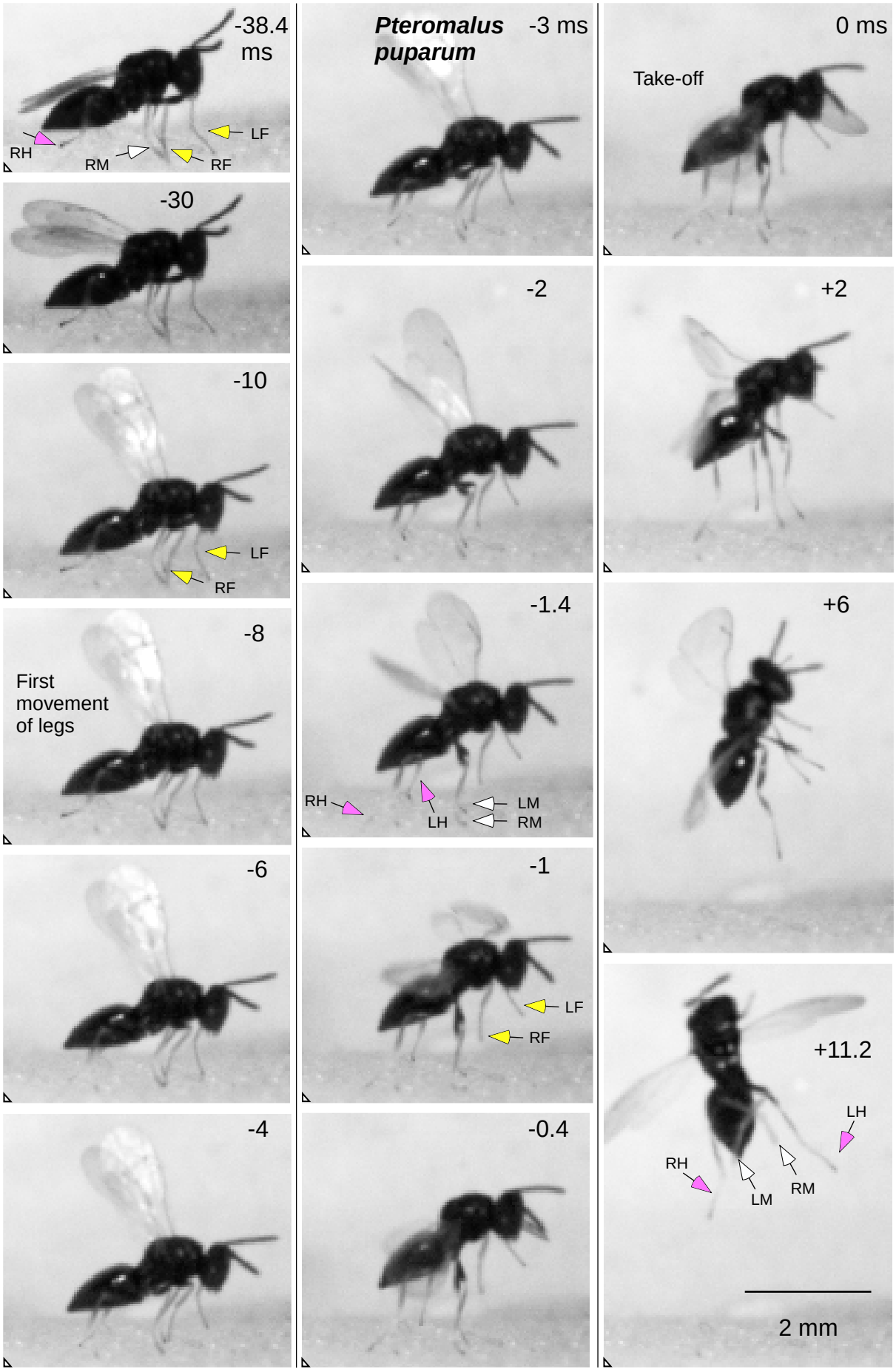

-38.4 ms
RH
RM
RF
LF
-30
-10
LF
RF
-8
First movement of legs
-6
-4
Pteromalus puparum
-3 ms
-2
-1.4
RH
LH
LM
RM
-1
LF
RF
-0.4
0 ms
Take-off
+2
+6
+11.2
LH
RH
LM
RM
2 mm

Figure 4

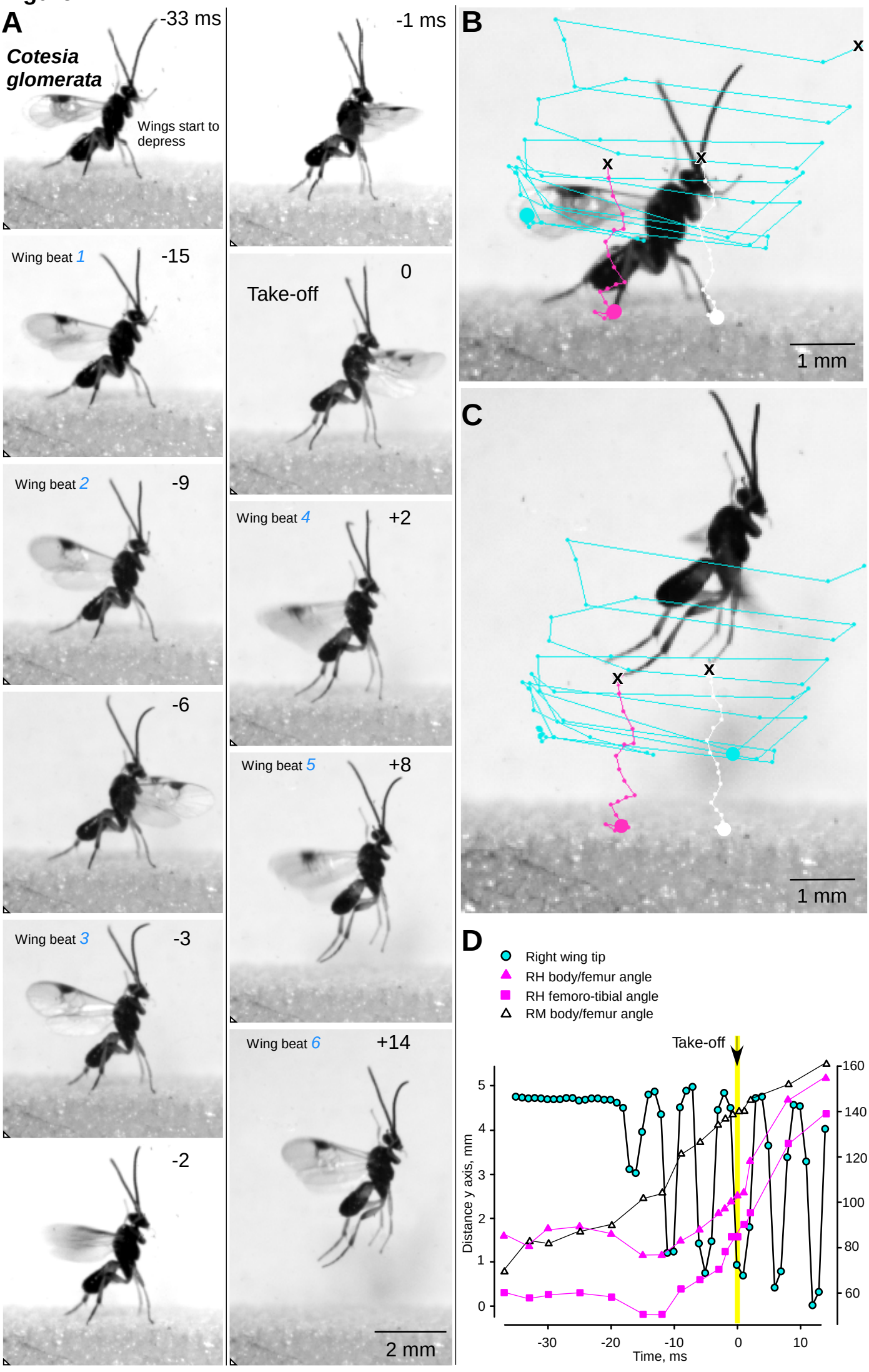
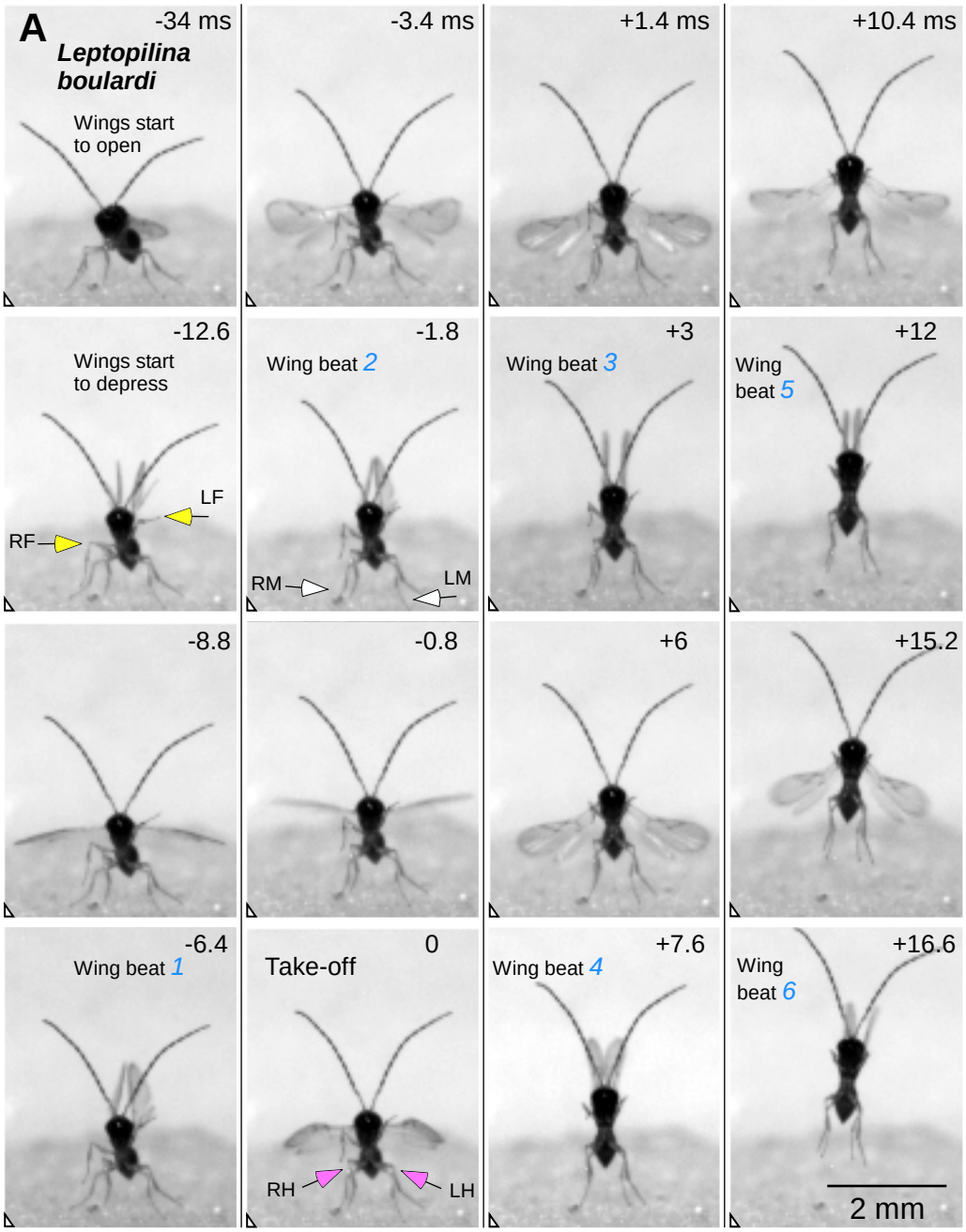
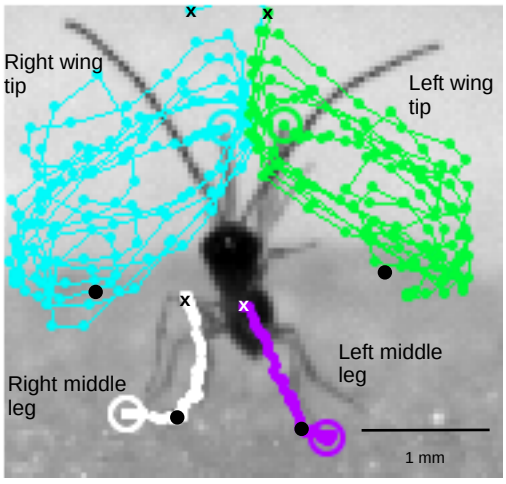

A
-33 ms
Cotesia glomerata
Wings start to depress
-1 ms
Wing beat 1
-15
0
Take-off
Wing beat 2
-9
Wing beat 4
+2
-6
Wing beat 5
+8
Wing beat 3
-3
Wing beat 6
+14
-2
2 mm
B
x
1 mm
C
1 mm
D
Right wing tip
RH body/femur angle
RH femoro-tibial angle
RM body/femur angle
Take-off
Distance y axis, mm
Joint angles, degrees
Time, ms

Figure 5


A
-34 ms
Leptopilina boulardi
Wings start to open
-3.4 ms
+1.4 ms
+10.4 ms
-12.6
Wings start to depress
LF
RF
-1.8
Wing beat 2
RM
LM
+3
Wing beat 3
+12
Wing beat 5
-8.8
-0.8
+6
+15.2
-6.4
Wing beat 1
0
Take-off
RH
LH
+7.6
Wing beat 4
+16.6
Wing beat 6
2 mm


B
Right wing tip
Left wing tip
Left middle leg
Right middle leg
1 mm

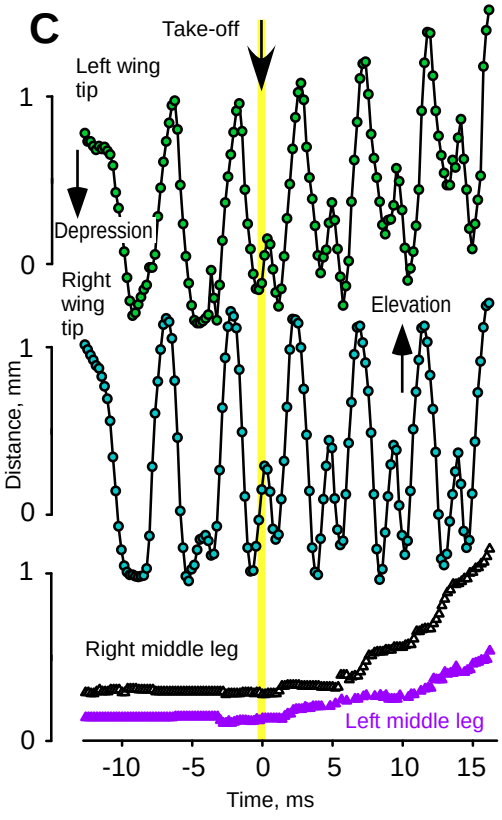

C
Take-off
Left wing tip
Depression
Right wing tip
Elevation
Distance, mm
Right middle leg
Left middle leg
Time, ms

Figure 6

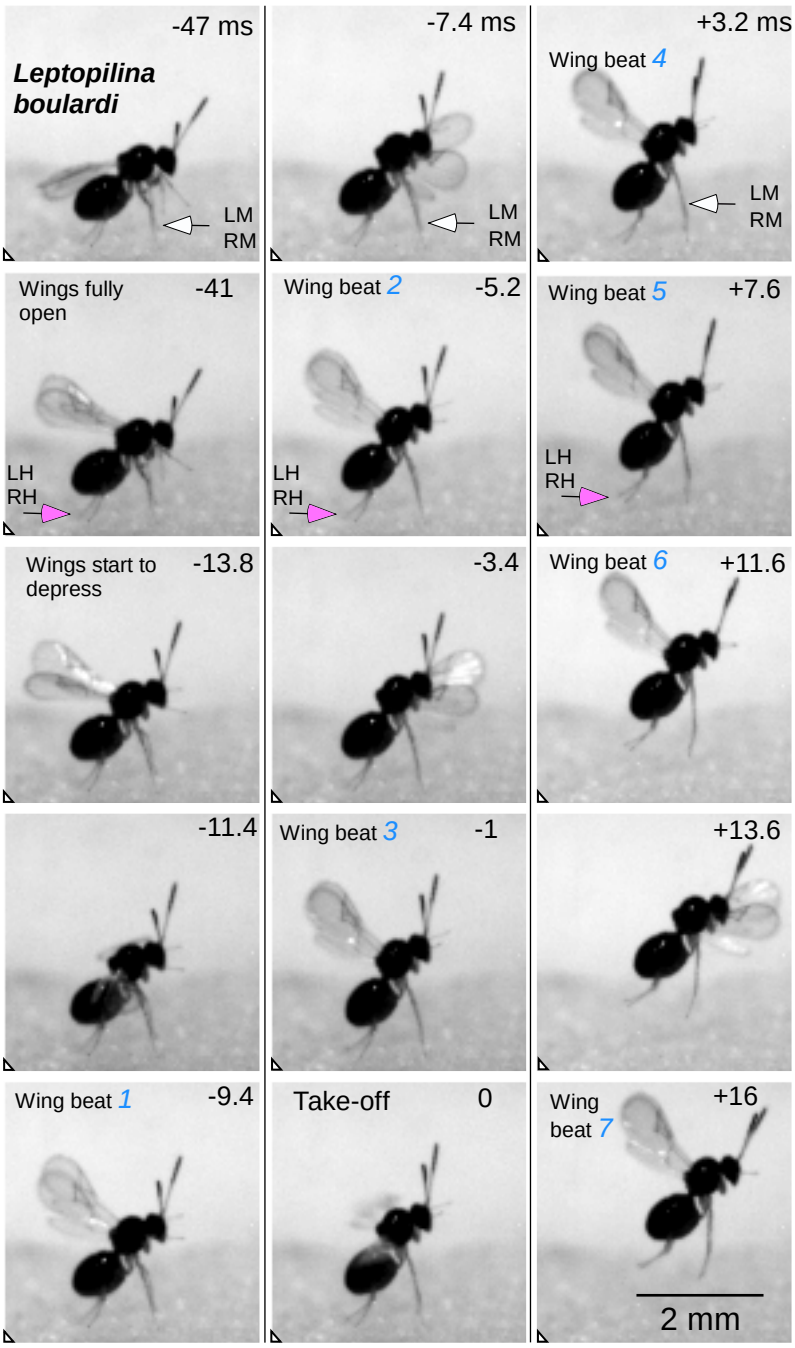
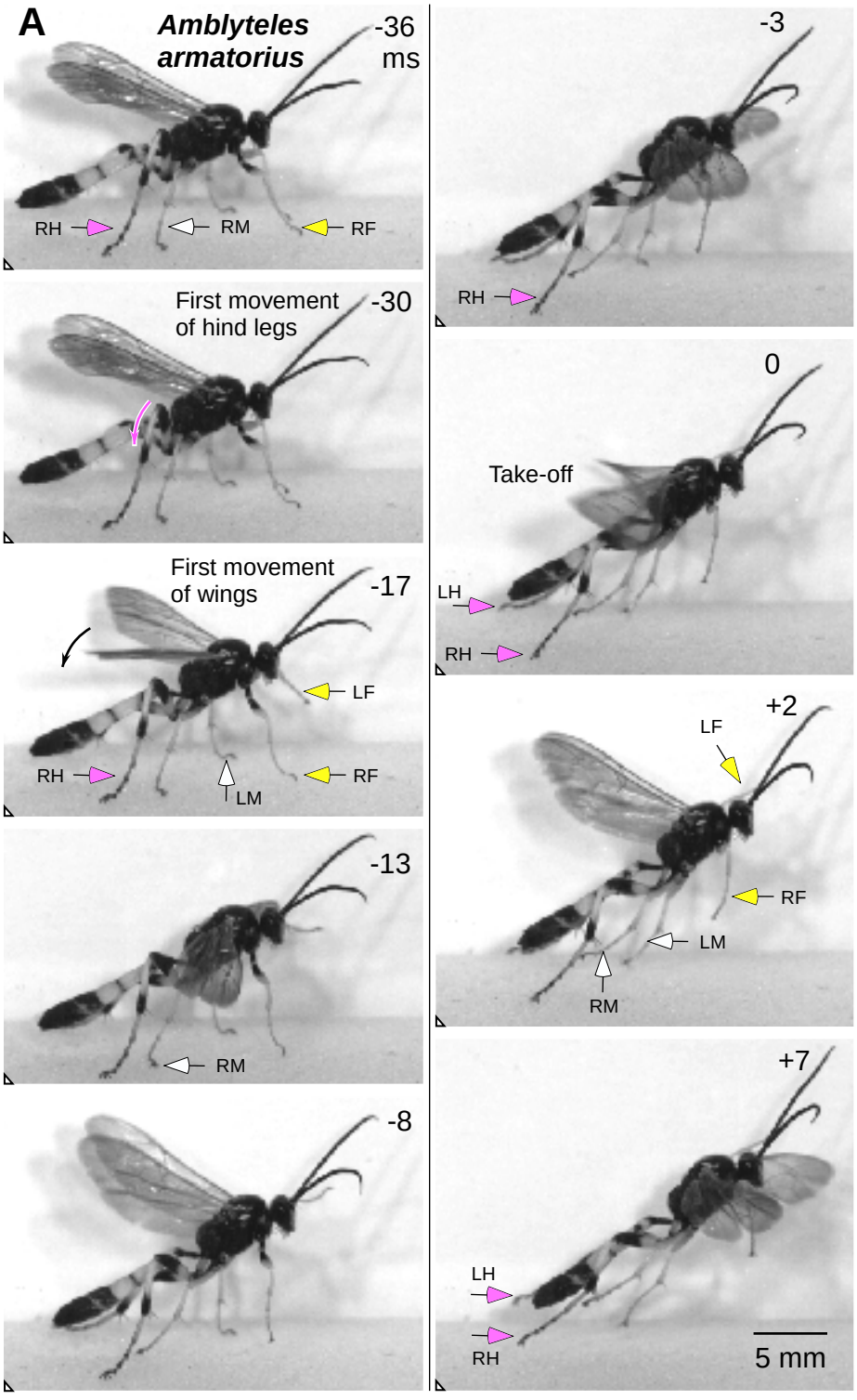

-47 ms
Leptopilina boulardi
LM
RM
-7.4 ms
LM
RM
+3.2 ms
Wing beat 4
LM
RM
Wings fully open
-41
LH
RH
Wing beat 2
-5.2
LH
RH
Wing beat 5
+7.6
LH
RH
Wings start to depress
-13.8
-3.4
Wing beat 6
+11.6
-11.4
Wing beat 3
-1
+13.6
Wing beat 1
-9.4
Take-off
0
Wing beat 7
+16
2 mm

Figure 7


A
Amblyteles armatorius
-36 ms
RH
RM
RF
-3
RH
First movement of hind legs
-30
0
Take-off
LH
RH
First movement of wings
-17
LF
RH
RF
LM
+2
LF
RF
LM
RM
-13
RM
-8
+7
LH
RH
5 mm

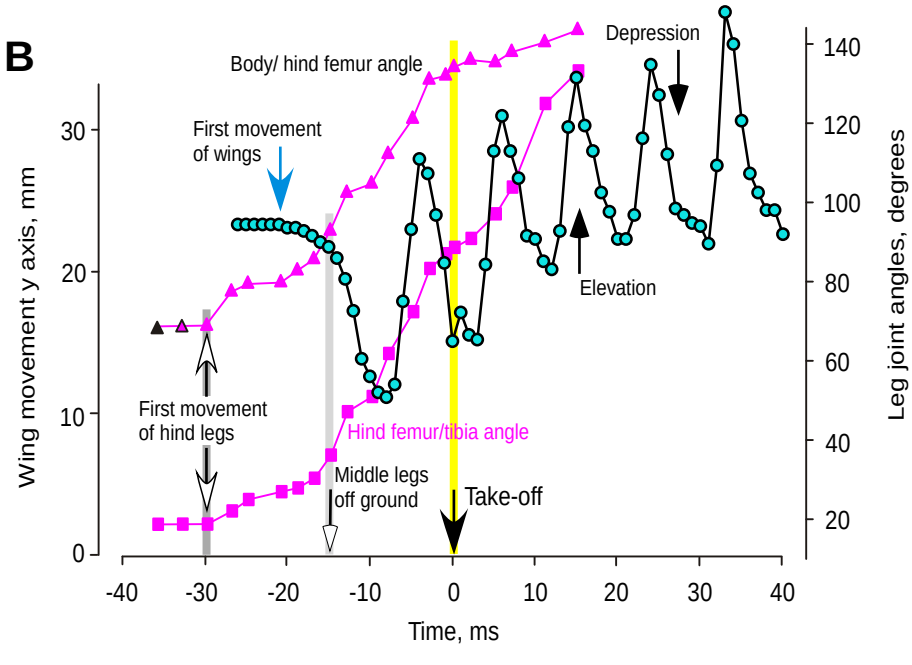
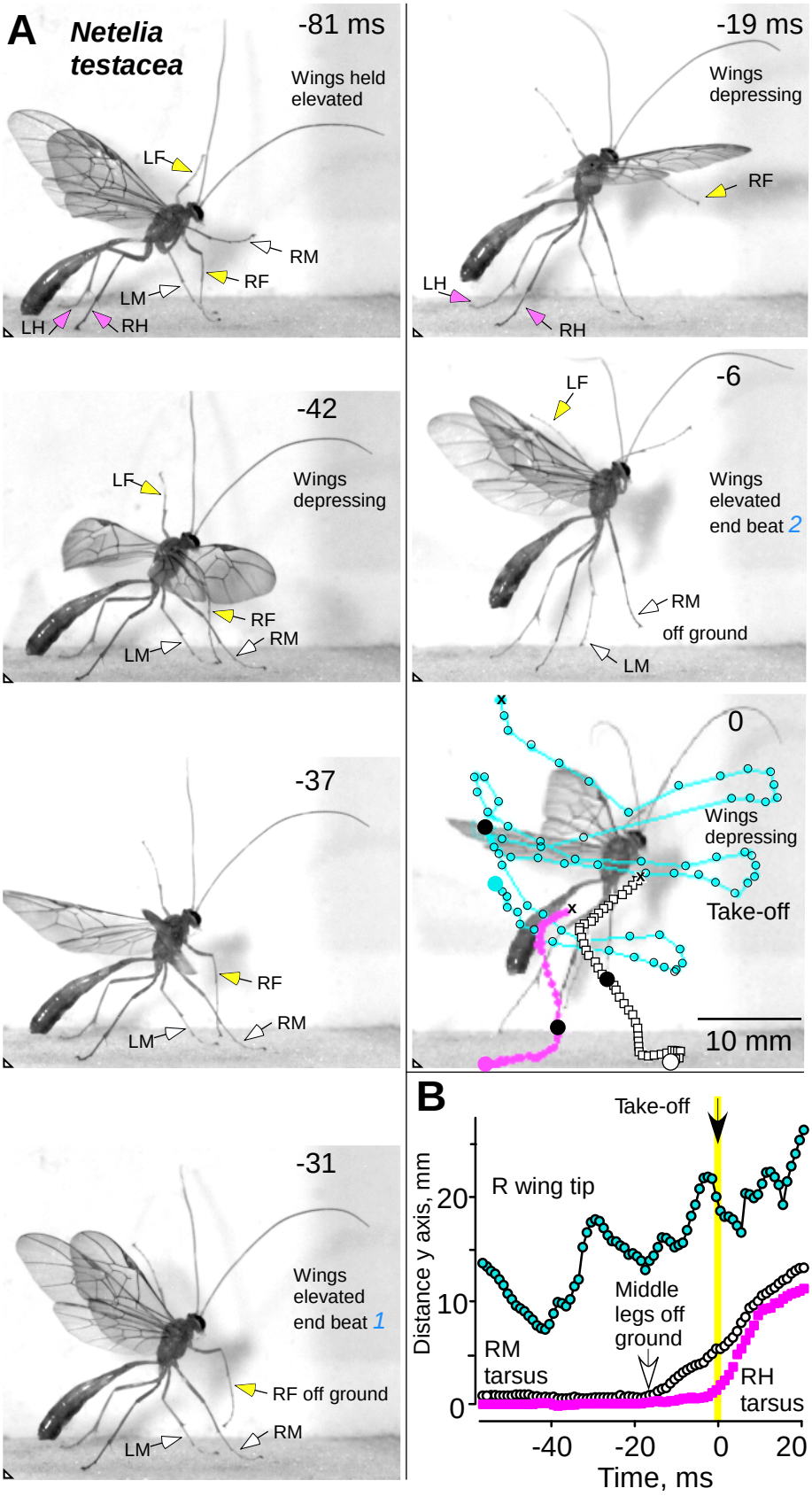

B
Body/ hind femur angle
Depression
First movement of wings
Elevation
First movement of hind legs
Hind femur/tibia angle
Middle legs off ground
Take-off
Wing movement y axis, mm
Leg joint angles, degrees
Time, ms
0
10
20
30
20
40
60
80
100
120
140
-40
-30
-20
-10
0
10
20
30
40

Figure 8


A
Netelia testacea
-81 ms
Wings held elevated
LF
RM
RF
LM
LH
RH
-19 ms
Wings depressing
RF
LH
RH
-42
LF
Wings depressing
RF
RM
LM
-6
LF
Wings elevated end beat 2
RM
off ground
LM
-37
RF
RM
LM
0
Wings depressing
Take-off
10 mm
-31
Wings elevated end beat 1
RF off ground
RM
LM
B
Take-off
R wing tip
Middle legs off ground
RM tarsus
RH tarsus
Distance y axis, mm
20
10
0
-40
-20
0
20
Time, ms